**FTS-CDC-EPO**

**Moderator: Denise Korzeniowski**
**February 15, 2006**
**12:00 pm CT**

(Wendy Zacowits): Hello and welcome to our teleconference Molecular Epidemiology of Noroviruses.

This is (Wendy Zacowits), bioemergency response training coordinator at the Arizona Department of Health Services in Phoenix, Arizona.

Today's teleconference is being hosted by the Arizona Department of Health.

Just a few program notes before we begin the program.

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Again, welcome and thank you for joining us. We have over 50 sites from across the United States listening to this teleconference.

Today's speaker is Dr. Steve Monroe. Dr. Monroe received his Bachelor of Science in Biochemistry from the Iowa State University in 1976. He received a PhD in Molecular Biology from Washington University in St. Louis in 1983.

And in '87, he began working at the Centers for Disease Control and Prevention, where he focuses on viruses associated with gastroenteritis.

In 2004, he was appointed associate director for laboratory science. Currently, he is the acting director of the Division of Viral and Rickettsial diseases in the National Center for Infectious Disease branch at the CDC.

It is my pleasure to introduce to you and to welcome our speaker, Dr. Steven Monroe.

Stephan Monroe: Thank you, (Wendy), and thanks to all of you in the audience who I can't see and I can't hear, but I'm assuming that you're out there. And thanks to Denise for setting up the conference and the other folks at the NLTN.

This is the second one of this sort of teleconference, video conferencing that I've done and although it's a little bit impersonal, I think it ends up being a good way to get the information out.

And I do encourage you if we have time at the end if I don't run on too long to ask questions. We've numbered the slides so that hopefully, those of you looking either on your screen or at hard copies will be able to stay in place.

So moving on to Slide 2; one of the things that I was told when I first did a teleconference is that because the audience can't see you, it's nice to include a picture so they have some sense of who you are and this is the picture of me

taken near Williams Lake in New Mexico back last June when my wife and I were doing a trial run of the empty nest.

So what we're going to talk today are about foodborne viruses, and in particular Noroviruses. But to set the background for why this is an important issue, these are data that are taken from a review that's a little bit dated by (Paul Meet), et al.

I'm looking at the role of viruses as the cause of sporadic foodborne illness. Sorry, we're on Slide 3 now. There should be two pie charts.

So on the left is the chart of total cases of foodborne illness in thousands. (Unintelligible) by this estimate about 9.3 million cases of foodborne illness each year resulting from viral infections as opposed to about 4.2 million from bacterial infection. So viruses are more important as a cause of infections.

On the right, looking at hospitalization, it turns out that bacterial infections tend to be more serious and so, the best estimate is that something over 36,000 hospitalization each year are due to bacterial infections and about 21,000 due to viral infection.

The next slide, we're going to look at the role of pathogens in sending people to the emergency department.

And this is summary data of three site study that was done through CDC's Emerging Infections Program. As you can see there are something over 360 subjects were enrolled. Stool and serum samples were collected from all the people in the emergency department.

And then they were tested by a variety of means essentially the best available test for a number of bacterial parasitic and viral pathogens.

And (well), you can see up highlighted is that in fact noroviruses were detected in 30 of the cases or 20%, which is larger than any bacteria or any parasite.

Interesting aside in this study is that rotavirus was actually detected in - also detected in 12% of the enrollees. And that's unusual because this study was limited to adults over the age 18 and we typically think of rotavirus as being primarily an infection of young children.

Importantly, the data shown there in the table is based entirely on RT-PCR detection of virus directly in stool. We also looked at the sera to look for evidence of a sera conversion in noroviruses and found that an additional 12% of the people were positive when the serologic diagnosis was included.

So roughly a third of this people overall who reported to emergency department with signs of gastroenteritis as evidence of norovirus infection, either by direct detection of virus in the stool or by sera conversion.

So on the next slide, Number 5, turning now from sporadic illness to outbreaks of illness and this is summary data from CDC's Foodborne Outbreak Surveillance System, which for many years and many of you may actually be contributors to this system, for many years it was a paper-based system where people submitted their information from state labs to a central database at CDC.

And the criteria were - included had to be a laboratory confirmed case of foodborne illness. And what you can see is through the early 90s, there are

roughly 150 bacterial or foodborne outbreaks that were laboratory confirmed from bacterial causes and especially in the 90s, there was on the order of six or seven outbreaks that were viral. And roughly five of these per year were hepatitis A and maybe one a year was Norwalk virus just because of the cumbersome nature of the sera diagnosis that was done at the time.

And in the mid-90s, we and other started to develop RT-PCR systems for detecting noroviruses and started to then transfer this technology to state health department laboratory. And so there was this increase as you'll see from '96 through to 2000 of the number of viral outbreaks that were reported.

Important to note if you look at the difference between '97 and '98, in '98, the system was switched to an electronic reporting system and there was a concomitant increase in the number of outbreaks reported.

Where you can see is that from '98 through 2003, there are roughly 230 bacterial outbreaks reported per year. Where you can see in red is that the number of viral outbreaks increased to the point where in 2002, there was almost the same number of viral outbreaks reported as bacterial. And when you look more closely at the data, you'll find that in fact there still are about five outbreaks of hepatitis A report - foodborne outbreaks reported every year, but the remainder of those outbreaks was norovirus outbreaks.

And so this highlights the importance of noroviruses as a cause of outbreaks of gastroenteritis.

And then again, on Slide 6 looking at outbreak data, this is another study that was done through the Emerging Infections Program at three sites, where outbreaks of foodborne illness were specifically looked at using an active outbreak detection algorithm in each state so to - reporting by local health

department and then aggressive sample collection coordinated by the state health department including in most cases the use of in-home collection kit, so the patients didn't have to report to emergency room or a physician's office to submit a sample.

And then again, there was comprehensive testing for bacterial, viral, and parasitic pathogens.

And in this study with relatively small numbers, only 27 total outbreaks in the three sites over the one-year period, over half -- 52% -- were laboratory confirmed norovirus, which was larger obviously than any of the bacterial pathogen.

And importantly in this study, almost a quarter -- 22% -- of the outbreak remained with an unknown diagnosis even though the best available methods were used for testing.

So, based on this and because there's a long-standing history -- so Slide 7 now -- of people who experienced with bacterial foodborne outbreaks, I refer the virus as the other enteric pathogen. And there are some important differences between viruses as a cause of foodborne outbreaks and bacteria.

First thing is for viruses, and this is true for either hepa A or for norovirus. So there's no replication outside of the human and so there's no animal reservoir unlike, (say, the) (unintelligible) on Slide 7. The pathogen is not replicating in the animal.

What that means is we have to directly detect the virus in the clinical environmental samples. And in this case, detection of the virus is equivalent to adulteration of the product, that is, if there is norovirus or hepatitis A on the

raspberries or whatever, then they shouldn't be there. The products been adulterated, whereas with meat products, you know, you may have some pathogens that are there. It's a normal part of the flora.

So, in contrast of the - not seeing any replication outside of the human host, there's very vigorous replication inside the human host. And so, it takes in many cases of very low infectious dose for noroviruses and estimated the ten particles are enough to cause infection.

And you generated very high yields so that a person who is infected with (ten) virus could excrete (in a total) during the time of their illness over (ten to the tenth) infectious particles out the other end. So, a little bit in can create a lot coming out.

One thing to remember about viruses, is that they are relatively difficult to disinfect compared to most bacteria, particularly these enteric viruses, hepa A and noroviruses. So, where I look at it is they're small in the inside and hard on the outside kind of like an M&M and makes them more problematic in terms of environmental persistence and disinfection.

So although we're going to focus on noroviruses, I wanted to quickly review some of the other viruses of gastroenteritis and to update you on the taxonomy of noroviruses.

So, those viruses have been clearly associated with gastroenteritis include rotavirus, which is primarily a disease of young children in which you may be aware just last week or perhaps the week before the FDA has once again licensed a vaccine for rotavirus for use in the US.

And in the next week or so, the ACIP, Budget Committee on Immunization Practices, will be meeting to decide what recommendations to make for the use of this vaccine.

Adenovirus is primarily Group F, Types 40 and 41, have been associated with diarrhea. Astroviruses have been associated with gastroenteritis primarily in young children, and then caliciviruses.

And coltiviruses, the terminology here has gone through an evolution over the years. They've recently been separated into four genera, two of which cause illness in human. Most common of which is the norovirus, formerly called Norwalk-like virus (more on structured) virus. We abbreviate this (NOV).

The other genus that has viruses that cause illness in human are the Sapoviruses formerly called Sapporo-like viruses or classic human caliciviruses because of their classic appearance in electron microscope.

These tend to cause illness primarily in children not so much in adults, but CDC investigated several outbreaks in adults that have been associated with Sapovirus.

Then there are number of poorly characterized viruses, corona viruses, (picovirnoviruses), and others that maybe a cause of gastroenteritis in humans. In many cases, they've been clearly demonstrated to be a cause of diarrhea in animals but has not been unambiguously associated with illness in humans.

And then I've mentioned some of this on Slide 9 now that the current taxonomy in the family Caliciviridae, the viruses that we're talking about now are in the genus Norovirus.

The type species of Norwalk virus isolated from or not isolated but detected in samples from an outbreak in Norwalk, Ohio, occurred in 1968.

The other two genu that I didn't mention because they don't cause human illness are the Vesiviruses and the (Lego-viruses).

So we've talked about the importance of viruses versus bacteria, so what's the relative role of different viruses as a cause of gastroenteritis in humans.

And so on Slide 10, this is study that was done by (Louis Pangetal) of children who are actually enrolled in a rotavirus vaccine trial, and so this is looking at samples from the placebo group are the kids who did not receive rotavirus vaccine.

Again, the largest chunk of the pie is negative, meaning there was no virus detected. But in this study, 19% of the kids had norovirus directly detected in their stool with 1% having mixed infection with Noro and Rota, 24% infected with Rota.

So in this study, a total of 20% of the children who were symptomatic with gastroenteritis at norovirus detected in their stool.

This is important because historically people have thought that Norwalk virus was primarily or was really a virus of adults and was not an important cause of diarrhea in children that rotavirus (was what) much more important.

What this data shows is that while rotavirus is clearly the most important cause of diarrhea in young children, noroviruses are close, second.

So moving on to Slide 11 to sort of set the stage; so what's been referred to as the diagnostic gap. So in noroviruses are such a common cause of illness, why are they so rarely diagnosed.

And the problem has been several, I mean that sporadic cases aren't reportable, outbreaks were rarely investigated, samples aren't collected. You can't make a diagnosis without a sample. And to this day, there's no clinical or commercial labs that are routinely testing for noroviruses in the US. There are a couple of commercially available kits now for detecting norovirus, but to my knowledge, none of this are available for sale yet in the US.

And this is (unintelligible) in part because there's no cell culture for small animal model for propagating these viruses, and so being able to make this sort of antibody reagents that are typically used to set up diagnostic (alignment).

And so that brings us to the molecular side of things and while we've taken the approach of using RT-PCR as a frontline diagnostic. And just to set the state a little bit, this is on Slide 12 now this is the genetic map of Norwalk virus as the prototype strain of this genus.

With a small single standard RNA virus, total length of the RNA is 7,654 base pairs and there are three open reading frames or ORFs, ORF 1, 2, and 3.

And ORF 1 encodes the non-structural proteins that are used to - as part of the replication of the virus. ORF 2 encodes the single major structural protein that's found in the shell around the virus. And ORF 3 encodes a minor structural protein that's found in the virus but at much smaller amounts than the protein encoded by ORF 2.

And over the years, we and others have developed several different PRC targets for detecting noroviruses, typically focusing on regions that are highly conserved.

And the one that's here marked as Region A of the CDC primers or the EU primer on the other one, targets are region of the RNA polymerase that's highly conserved. Subsequent to that, we've developed primers that we call Regions B, C, and then in collaboration with (Dionne Vignette), who is then at the University of North California, Chapel Hill, and as just last week moved to join us here at CDC Region D down in ORF 2 in the (unintelligible) region.

And while some of this evolution has taken place over time as to which primers that we've used, in part the changing from one type to another has been based on our increasing knowledge of the usefulness of different PCR targets and the kind of epidemiologic information that we can derive from them.

So on Slide 13, the question, why do we use an approach where we do PCR and then sequencing?

And for us the important thing about sequencing is it gives us a confirmation that our PCR products are actually what they think they are. Some of the PCR products that we used do not distinguish genu groups well and so we use sequencing to cluster viruses into groups.

But the other thing that sequencing has proven very useful for is to clarify the epidemiology of transmission in various outbreaks and I'll give a couple examples of that as we go forward.

I always have to put in this one caution now on Page 14, a picture of the elephant and I'm not sure how many of you are familiar with the parable The Blind Men and The Elephant, but the parable is something like, you know, the six blind men were asked to describe the elephant and of course depending upon which part of the elephant they touch, they had a very different view of what an elephant looks like.

I'd say that the molecular subtype thing is a bit like this parable. And that is, that you get an entirely different picture of what you are looking at depending upon what part of the beast you're examining. And it's a limitation of only using a small fraction of the virus for the sequencing.

And this is the graphic example of that on the next slide, Slide 15. So on the left is the genetic tree or (dander gram) drawn using sequence information from outbreak strains and using the PCR target that we called Region B.

On the right is information from many of the same strains using what we called Region C. I'll remind you that Region B is in the RNA polymerase and ORF 1; Region C is in the captured protein.

Couple of points from this slide; first thing is, Region C primary set that we've currently developed only amplify strains from Genu Group II not strains from Genu Group I and so in the tree on the right, there's no strains representing Genu Group I except those reference strains for which sequence information is available from (gene bank).

But the important point is if you look - focus first on the right side, in the areas that are shaded in, these are strains that fall into what we defined as genetic clusters. And as you can see, the clusters are fairly clearly defined

with Region C. And you see Genu Group II Cluster I, Genu Group II Cluster X, et cetera.

You follow the arrows and across to the left side, the sequence is from Region B, the exact same viruses. What you see is that there are - while there are some separations of strains in Region B, they tend to be grouped together into big clumps that are not easy to resolve. And in particular, if you look at the - near the bottom, at Genu Group II Cluster VI and VII where they're clearly distinguishable by Region C sequence. And they're sort of all mix together by Region B sequence.

But that doesn't mean that the Region B sequence is aren't useful but you get a different answer about how related strains are depending upon which part of the genome you're looking at.

In the next slide, Number 16, this is a tree the sort of standard tree by which all others are measured, this is a genetic tree looking at the complete captured protein for the open reading frame 2 and looking at amino acid sequences instead of nuclear type sequences, what we've been able to do is define different clusters that are well resolved by this analysis where the clusters differ by at least 20% in their amino acid (distance).

That's a pretty big difference between viruses. And it's probably the reason why people can be infected with different strains of virus and there doesn't appear to be much of the way in cross-protection between strains within a genu group. There may perhaps be cross-protection between genu groups.

The difference between genu groups, say, Genu Group I classic Norwalk virus, in Genu Group II classic Snow Mountain Virus and so in the order of 45 to 55% of the amino acid (unintelligible), the amino acid level.

So these are very different viruses.

(What) I've highlighted in yellow and I don't know how well that shows up (unintelligible) black and white hard copy. But there are three clusters of virus - excuse me - two of them in Genu Group III, Genu Group III Cluster I and II, which are found only in animals. And the interesting one is Genu Group II Cluster II, which is represented here by a strain called Sw918. That virus was found in pigs in Japan and what's interesting is that so far that virus has only been found in pigs but it clearly falls into Genu Group II with lots of other human viruses.

And so it may actually represent a sort of reversed (diagnosis), where the virus was originally a human virus and then was transmitted to pigs and now maybe maintained in the pig population.

So on Slide 17, (unintelligible) talk now about the epidemiology of norovirus infections focusing primarily on outbreaks.

And a couple of characteristics: one is that we know the virus can be transmitted through multiple routes, there's foodborne outbreaks, water-borne outbreaks, person-to-person spread either in institutions or secondary spread to household contacts of somebody infected somewhere else.

And often there are mix modes where an outbreak, say, in a nursing home might start out as a foodborne outbreak but then be spread person to person.

The other thing in terms of the public health response is, these outbreaks can be very difficult to control because, first of all, they're very common and so they don't generate a lot of interest among public health staff.

As I mentioned, there's very low infectious dose. So it takes relatively little contamination for somebody to get infected.

We know that viral can occur for a long time by PCR now up to 20 days at least after somebody is infected. There is still (detecting) virus that's detectable by PCR.

And people can be shedding virus in the absence of clinical illness either before they're sick or they can never be sick or after they've recovered from illness. And so it's sometimes difficult to say for food handler to realize that the person might actually be shedding virus.

As I mentioned, the viruses are hardy and so they persist in the environment. They're resistant to many common disinfectants. And (unintelligible) to look, the strain diversity means that there's probably not good immunity from one genu group to the other.

So now I'm going to take you through several sorts of case studies of outbreaks that are associated with noroviruses, focusing on those that have occurred from foodborne exposures on Slide 18.

And there's a concept in food safety of trying to maintain safety from farm to table. And although most of the contamination with viruses occurs downstream, closer to your table, there is contamination on the farm. And the one classic example, this is contamination of oysters in the oyster bed.

But more recently, there are several examples of contaminations of raspberries. There were contaminated in the fields with norovirus and then subsequently lead to human disease.

So I'm going to focus on a couple of outbreaks here where the contamination was closer to the table. And the ones in bold or in yellow are the ones that we'll go through in case history.

So on Slide 19, it's an outbreak. It's a little bit dated now but it's one of our success stories, so I always come back in talking about it. So this was a university dining hall in Texas on March of '98. There was acute illness that was associated with epidemiologically with either lunch or dinner from the dining hall deli bar. It was a case where students had a choice of different menu options and if you went to a deli bar and ask to have a sandwich made for you, you are more likely to be sick.

One of the things this points out is the number of the stools that although we always thought of Norwalk infection as being relatively mild, 23 students were actually hospitalized from this outbreak. Of the 18 stools that were collected and were tested for norovirus by PCR, 9 were positive for norovirus.

And it's not uncommon using the standard PCR techniques that somewhere between 50% to 70% of the stools are positive but rarely are 100% of the stools positive.

In this case, there was a food handler who had a child who was also ill. The child was PCR positive. And interestingly in this outbreak, once the deli meats were implicated, (Kellogg Schwab), who was then working with (Mary Estes') group at (Bailer) now at Johns Hopkins in Baltimore, took some of the hand samples, washed off the surface to try to extract the virus, tested PCR, found that they were positive by PCR and importantly and this is where the sequence analysis comes in, he sequenced the PCR product he got in his lab. We sequenced the PCR product from the stools in our lab and the sequences

were 100% identical confirming the epidemiologic link between the food and illness in the patients.

And then a little bit closer to us here in Atlanta. This is again is little dated but, you know, (Gwyneth) County is just the next county over from us. There was an outbreak that was associated with contaminated products that were purchased at a large grocery store.

So on Slide 21 now; and so these were two gastroenteritis that was associated with eating especially cakes that were prepared in a grocery bakery. So, if you went to the grocery store and just picked up a (sweet) cake and went home and ate it, you were fine. But if, say, you went to the grocery and picked up a cake and then had then write, you know, "Happy Valentines Day" on it with the red frosting, then you were in trouble.

And so on at the investigation of this, they found 153 cases out of 195 people who had attended 38 events and the events might be, you know, a mom went in and got a big chocolate chip cookie and had "Happy Birthday" written on it and took it in to the school class or a cake or whatever.

And in looking at the association, the decorated cooks were highly associated with the illness, odd ratio of 22.2.

And sure enough, when they went and did the investigation, one of the food workers admitted to being ill while she was frosting cakes. And in this case, even though there were 153 people who are identified that were ill, we only receive 15 stool samples for testing but they were all positive by PCR. Three of the products were identical and as often the case when we try to detect virus directly in some of the frosting, it was negative by PCR, probably reflecting the low level of contamination.

This is another outbreak now in Slide Number 22. It shows the interesting mechanism of transmission. So this was an outbreak that was in - actually in the Netherlands. Two hundred fifty people sick, and the illness here was associated with eating lunch rolls, which were small sort of finger sandwiches that could have different kinds of meats inside of them.

And again, during the interview of the deli that was associated with this, the baker admitted that he had vomited in the sink but then he cleaned it up and then had gone about hand-slicing the rolls and putting the different meats and things inside the rolls.

Again, 24 of 27 stool samples were positive by RT-PCR. They all had identical sequence and as did the samples from the baker and his family. But in this case, no food samples were available for testing to make the definitive link between the food and the cases.

So moving on - excuse me - to Slide 23, this is the so-called epi curve of that outbreak, where you can see is - but they were served and then roughly 33 hours later, people became ill (unintelligible) the median incubation time was 33 hours.

And this epi curve showing the timing of cases with the sharp rise and sharp fall is pretty classic for a point-source foodborne exposure. In this case, the buffet was only available for an hour or so and so all the people were exposed at the same time.

And then as a sort of final example of foodborne illness, this is a wedding cake associated outbreak from Massachusetts in April 2002. This outbreak is just been written up, it hasn’t come out yet but will be coming out shortly.

So again, there was acute gastroenteritis associated with eating wedding cakes and from what I understand, the bakery involved here was written up in Bride magazine as one of the premier places to get your wedding cake from.

So again, when the epidemiologists do the investigation of 12 different events that occurred over a single weekend, 332 out of 850 were ill. And what they found was that there were actually were 46 events where cakes from this bakery were served during that weekend.

And so if you project that attack rate, there will be over 2,700 people who would potentially have been ill from eating contaminated cakes. Again, the wedding cakes were highly significant as the vehicle infection. These food workers admitted to being symptomatic.

And again, using PCR products from different weddings and sequencing those and showing that all the PCR products were identical sequence, provided the molecular link to show that these outbreaks were all related.

And so now moving on from sort of looking at this individual case studies to thinking about multi-state outbreaks and how this can occur and how we can go about trying to track this down.

One of the problems is that because of norovirus is so common; we can't easily identify multi-state outbreaks just because people in two different states are sick, even if they're confirmed to have norovirus.

And so how would a virus spread from one state to the other, well, you could have a sort of point-source spread where people are infected at one place and

then travel to another area, either at a conference or they're on a cruise ship and then they leave and go home and spread the virus.

The other thing you can have is that a contaminated product could be distributed over a large area, and so we know from the oyster outbreaks that the oysters have - contaminated oysters have been distributed and have resulted in outbreaks in multiple states. And the raspberry experience in Europe showed that a contaminated product can be distributed and cause outbreaks in multiple places.

Now I want to turn our attention just for a little while to cruise ship outbreaks and these are still going on, but it was really - there was a time in 2003 - 2004 when there was a big increase in cruise ship outbreaks and they were making a lot of news.

And one of the features is that for one thing, you can have multiple points of exposure on the cruise ship, so it can be brought on by passengers. It could be brought on by crew or it can be brought on by contaminated food or water.

And then you have the potential because you have so many people in close quarters that you have a potential for this mixing bowl where you could have multiple strains circulating at the same time. And this is again as we'll see an example of where sequencing to actually do this molecular epi can help to distinguish a single event from multiple contamination event.

So now we're looking at Slide 27. I'm going to go through a couple of cruise ship outbreaks where we were involved with the investigation.

Excuse me.

So this is again a curve showing the - epi curve showing the number of cases in either passengers or crew by onset date. And there's color coding here that you may not be able to see in the black and white. But the earliest case in the 10th of October, actually where two different viruses that were detected in that case and then there was - that was in a crew member.

And then there was a new crew started and with the onset of this peak of onset around the 13th of October, this is 2003. And number of cases, we got stool samples. They all ended up being a Genu Group II Cluster IV strain, which we in first found in many outbreaks both on land and on cruise ship. And so we referred to this as the common strain and we gave it the name of Farmington Hills because that was the first place where we had identified this strain, actually back in March of 2003.

So to summarize the experience of this ship - Cruise Ship A, Slide 28, it's a five-day Caribbean cruise. The outbreak level reached 5.8% of passengers, 7.8% of crew, typical symptoms of norovirus infection.

And what we found was that there were multiple strains on this ship. And in particular that there was this Genu Group II-IV strain that was common at the end of the cruise.

Now looking at Cruise Ship B, and this is an interesting story. So we're on Slide 29, I suppose.

In this case there were - well, scheduled to be four-week long cruises and on Cruise 1 and Cruise 2, that peaks of illness when we did the testing, we found that it was this common Farmington Hills strain of norovirus. There was a lot of press about this and the cruise ship company decided, make the decision that in early December, again 2003, they decided to cancel the cruise and do a

thorough steam cleaning of the ship, all the bathrooms, all the common surfaces, put the ship back into service at the following week.

What we found was that sure enough, there was another peak - another smaller outbreak. But again, when we did the sequencing and looked, we found three distinct sequences circulating amount the passengers on that subsequent cruise. One of which is GII-IV that shows in red, if you have a color version, was identical to that which was found on the two previous cruises.

On the next slide is summary of this. The interesting thing is on the first two cruises, it was a single strain that was detected in both passengers and cruise, but then on the - crew - but on the third cruise, we found three different strains that were detected including the GII-IV strain from the previous two cruises.

But this is actually consistent with a transmission and introduction model, where - while there may have been persistence of the virus on the cruise ship because the same virus was found in the Cruise Number 3, it also points to the fact that there likely was reintroduction of virus by passengers getting on the ship as we found these two new sequences.

And in fact because the Genu Group II Cluster IV Farmington Hills virus was quite common on land at the same time, it’s also possible that the cleaning of the ship was absolutely efficient and that the GII-IV strain that came on wasn’t left over the ship but actually was reintroduced by a passenger.

And then I’m going to go quickly through a couple of other outbreak scenarios here.

This is a case of an outbreak in Las Vegas, Nevada, where there was actually a group of sushi chefs came together or participating in a conference. There was an outbreak among the hotel patrons. Three of the chefs became ill during their visit. One of them returned to Hawaii and then the restaurant where he was at, patrons and the waitress became ill after eating the sushi that was prepared by one of the chefs.

And so this shows how a person can be infected in one place and then transmit the virus to another place.

In this case, again, using the molecular epi on Slide 32, we (were on a show) from nucleotide sequence that the strain that was in the sushi chef, the strain that was in the Hawaiian restaurant patrons and the strain from several persons who were also affected at the same hotel in Nevada had identical sequence showing that it was the same strain that was transmitted from place to place.

Quickly now, I'm on Slide 33. I'll just skip through this. This is the raspberry case. Again, as I said, this was both in Canada and in Europe that several clusters of illness were associated with raspberries and in this case much like the deli ham from the Texas outbreak not only where viruses detected in the patients but virus was detected in the raspberry.

And on Slide 34, summarizing, it was an identical strain that was found in the cases within in the raspberry puree confirming the epidemiologic link.

And what was found is that there were similar outbreaks were traced to frozen raspberries in Finland, in France. And it's probably has to do with the irrigation practices that were used while the raspberries were being grown.

And this has been written up and reported by the group (deck).

So now I'm going to talk a little bit at the very end here about how we can try to use sequence information to link outbreaks. And this is - if many of you are familiar with probably the (Pulse Net) model of can we use sequence information to link outbreaks even when there's not an obvious epidemiologic link, Slide 36.

And so what we did is look in the maps on Slide 37. At the appearance of this Genu Group II Cluster IV virus, over a two-week period in February 2004. And so what we noticed was that there were three outbreaks that occurred over a relatively short of time, two weeks, in a relatively small geographic area, that is Georgia and Kentucky.

The settings were quite different. It was nursing home outbreak in Kentucky. And it was a - or a conference outbreak in Georgia and the other one was a nursing home outbreak in Georgia.

And although these viruses had identical sequence, we couldn't find any epidemiologic link that would suggest that there was a common food that was served in this places or is that there was a person who traveled from place to place or some other vehicle that would account for the appearance of the same virus in this three different outbreaks.

So although we have the molecular link, we weren't able to find the epidemiologic link.

And then again, looking at this GII-IV virus; also started to show up in a number of different outbreaks over a three-week period in January 2005. And so in this case, what tip us off was that there was a number of cruise ship

outbreaks, interestingly, some in the Caribbean, some in South America, on the Pacific Coast and then a nursing home in Alabama.

All was identical virus by sequencing of PCR products. All within a relatively short period of time.

But again, we were unable to find a definitive epidemiologic link, say, a crew member who was on one of these ships who transferred to another ship, something like that that would have been the sort of smoking gun to show that these outbreaks were - had a common source exposure.

So now sort of putting this all together, Slide 39. We definitely have examples where we have the identical virus circulating in different places. But unfortunately, we don't have a definitely epidemiologic link.

And so it (unintelligible) to think about the potential for linking outbreaks using molecular approaches. But so far we've been unsuccessful in being able to sort of go backwards, that is, to find the sequence link first and use that to uncovered an epidemiologic link.

And then I sort of alluded to this Farmington Hills strain and I'll talk just a bit about this. And it really started in late 2002. We noticed this sharp increase in outbreaks, both land and sea, single sequence type, Slide 40 now.

It was found to be predominant and we provisionally named the strain Farmington Hills because of the location where we had first identified the strain with this particular sequence pattern.

And in (unintelligible), I'm not sure - I guess it shows up on my - black and white version shows up okay. So the dates in yellow are the ones where

there's outbreaks during the early period, April to February 2004, the lighter strain, the lighter color on the black and white.

In looking at the settings, because people were referring to this virus at that time as the cruise ship virus and what we found looking to the analysis of 25 outbreaks over this period is that while a lot of them were on cruise ships, 36% (within) the piece chart on Slide 42, and the equal percentage of these were in nursing homes, 16% in school and 12% in what we would call food settings, restaurants, things like that.

So the one thing did appear to be common theme with these outbreaks is they tended to occur in places where there was a tight grouping together of people, cruise ships, nursing homes, schools. So we thought maybe there were something about the way this virus was transmitted that was peculiar.

And so one of the things we did was to compare the symptom profile of people who were infected with this virus to that of people who were infected with all the other strains that we had seen in outbreaks that were going on at the same time.

And what we found, a little bit disappointingly was that although there was a slight increase in the likelihood of having diarrhea, 87% versus 80% that was just barely statistically significant in the Farmington Hills outbreak, there was no difference - statistical difference in their frequency of vomiting, so 73% versus 76%.

One of our models had been that perhaps because this virus seems to be common in settings were people are tightly packed together is that there maybe an increased frequency of vomiting resulting from infection with this virus. But that turned out in our (announced) system not be the case.

Limitation of this study is that we were only looking at the small portion of the genu primarily Region B to do a little bit of sequencing in Region D. Because this was the predominant strain, it limited our ability to link outbreaks because many outbreaks have the same sequence.

And overall an issue dealing with these viruses - (and as I said) we have the one success story of being able to detect virus on the deli ham, Canadians have been able to detect virus directly in raspberries. We had some success in detecting virus in implicated water sources.

But in general, it's, you know, impossible to detect virus directly in the contaminated food or water. And so makes it difficult to directly link the implicated vehicle with the strain found in the stool samples of cases.

Going back to that - the study of the Farmington Hills virus and the analysis of the different strains. This analysis was just been published in the most recent issue of the Journal of Infectious Diseases. And in fact the cover of JID has a map showing the frequency of outbreaks by state that are reported in that study.

So concluding then the sort of take-away messages I hope you get from this presentation today on Page 45, norovirus is the leading cause of sporadic cases and outbreaks of acute gastroenteritis in adults and is an important cause of disease in children as well, secondary to rotavirus.

And so as our methods for detecting viruses have improved, the importance of viruses has - our understanding of the importance of viruses has grown.

It's not to say that bacteria are not important but to just say that we probably been underemphasizing the importance of viruses as a cause of acute gastroenteritis.

As we've seen from some of these examples, norovirus can be transmitted by multiple routes of exposure and different ways of contaminated foods or surfaces, and so it makes it challenging to try to dissect in an outbreak investigation exactly what the exposure was.

At any given time within a community and as we've seen even within a cruise ship, there can be multiple strains (could) circulating. And so genetic characterization of strains is, I would say, essential for both distinguishing and linking cases.

And as we've seen with the common strain, it's difficult if you have two people who are infected with the same virus and that virus is common throughout the country, it's difficult to find an epidemiologic link between those two cases.

So I often say that sequencing is a bit like a paternity suit in that it's much easier to show that things are different by sequencing and to prove that they had a common exposure by sequencing.

So, again, to the cruise ship where we had multiple strains in circulation, it's definitely consistent with a model where there were multiple introductions in that environment. (It said that) norovirus is so common that we cannot easily identify multi-state outbreaks without using molecular epidemiology.

Our future goal is rapid diagnostic assay, real-time RT-PCR. I didn't talk about that today but we have in fact developed a real-time RT-PCR assay for

norovirus. It allows for more rapid detection, more sensitive detection, but in order to do this molecular epidemiology, we still need to do the traditional PCR and sequencing in order to have the fine information to say, these two strains the same or are they different.

What we're trying to do is increase surveillance, which what that really means is we're trying to get out state and local partners to increase surveillance and we're trying to get increased strain characterization, which again what that really means is we're trying to get our state and local partners to do more of PCR and sequencing on their own.

And the last slide finally, of course, I didn't really do any of this work. I'm just here to tell you about it. This is an (Adams) and (Suzanne Bear) are the ones who did most of the sequencing data that's presented today. (Leslie Hadley) was involved with methods development as with (Amanda Newton). (Angie Trahio) has developed the real-time assay. (Dang Wee) and (Duping Zang) have done the sequence analysis and comparison stuff. (Joe Rizzo) was the lead of our epi group. (Lanai Brown) is the one who did the epi analysis of the symptom profiles of the different outbreaks. (Unintelligible) actually did the outbreak in the Netherlands that I described and his now the head of our epi activity.

And of course, we don't do anything here at CDC without the collaboration of state and local lab and epi folks like many of you who are on this call today.

That concludes my part of the presentation. We'll open it up for some questions now. I believe that…

Coordinator: Thank you.

We'll now begin the question and answer session.

If you would like to ask a question, please press star-1. You'll be prompt to record your first name.

If you - to withdraw a question, you can press star-2.

One moment, we'll wait for a question.

Stephan Monroe: And I would just say, if you're going to ask a question that's specifically refers to one of the slides, let us know what the slide number is so that we're all looking at the same page.

Coordinator: Yeah. I do have a question. I'll - I'm not sure I have the right name. Let me just introduce you.

Is it (Neil) from Maryland?

(Naomi Barker): (Naomi Barker).

Coordinator: Thank you.

Go ahead and ask your question.

(Naomi Barker): Steve, (unintelligible) this is (Naomi).

I'm wondering how do I get to your Web board. I'm having problems, so that I can actually look at the prototypes of the viruses when I do the sequencing.

Stephan Monroe: Right. And the Web board - (Lanai Brown), now (Blanton) was the one who was in charge of the Web board. She's actually like a number of people here. She has moved to our flue activity.

And I actually don't know who's running the Web board now.

(Naomi), I can try to send an email with that information. But unfortunately the - we had hoped to put in place a system that originally I called (Calici Net) using the sort of (Pulse Net) analogy. Eventually, it became called (ID-MEDS), which was infectious disease molecular epi database system.

It's become a victim of the IT funding shortfall within CDC that the system is almost ready for primetime but not yet ready. It hasn't passed the computer security procedures necessary for us to make it available to folks on the outside.

So we don't have the automated system that I'd hope we would have for people to submit sequences.

In the meantime, what people have done is to email sequences and then we've run the comparison against our database and return the results. There is an email box you can use, which is calicinet@cdc.gov.

(Naomi Barker): Thanks.

((Crosstalk))

Coordinator: Okay. (Further) questions, please press star-1.

Stephan Monroe: So I guess that mean that…

((Crosstalk))

Man: (Colorado Public Health Lab).

Coordinator: We have a question…

Man: My question is, could you elaborate on the relative sensitivity of the real-time assays versus conventional PCR?

Stephan Monroe: Sure. In our hands, the real-time assay and what we do was took some published assays and tweak them a little bit to make them more broadly reactive and to also pick up the Genu Group 4 strain. And what I can tell you is I - just yesterday, we heard the comments on - the reviewer's comments that we're sending back to the journal so that work should be coming out, hopefully within - probably a month or so.

So the real-time using synthetic transcript RNA, where we know exactly how many copies we put into the reaction, the real-time assay is able to detect on the order of 10 copies of RNA.

Depending upon which primer set we're using for the conventional PCR, the Region B which we've used for years and years is our frontline diagnostic PCR because it employs a mixture of primers and primers that have the (general season) (unintelligible) (inosine) at some positions to make them more broadly reactive.

We know that it's actually fairly insensitive and so that assay is on the order of 500 to 1,000 copies of RNA. So the real-time assay is on the order of 100 times more sensitive than the conventional PCR.

Some of the other PCRs, the Region C PCR because it's more specific only for the Genu Group II strains is actually more sensitive than the Region B PCR, but neither one of those are as sensitive as the real time.

So our current approach to outbreak diagnosis is to - if we have an outbreak, we still like to test about 10 samples from the outbreak. We'll test them by real-time PCR, maybe eight or nine of those would be positive by a real-time PCR.

The advantage of real time is that not only it tells you plus, minus, but it also gives you a feel for the relative connotation, although it's not strictly quantitative. You do get a sense from the (PT) values of strong positives versus weak positives.

And so from the strong positives, we select three of those to do conventional PCR and sequencing.

Man: Thank you.

Coordinator: Next question comes from (Dave) from California.

(Dave Snare): Hi, Steve. This is (Dave Snare).

Stephan Monroe: Hey, (Dave).

(Dave Snare): Hi.

I got a question about the pie chart on Slide Number 3 and 6. They both seem to indicate that the percentage of norovirus is - on the first one, fairly low and

on the second one 52%. And they may be calculated differently that we do our outbreaks but - and I think I'd say this literature also we're getting like 70% to 80% of those outbreaks that we test positive for norovirus.

Stephan Monroe: Right, (Dave), and that's - because you guys are better at looking than we are. Now, yeah, the figure on Slide 6 is looking at outbreaks as the unit of measure, where the 52% where positive.

The figure on Slide 3 is actually estimates of total illness for foodborne where most of it is estimate, say, for the cases on the left, 62 million cases where by extrapolating what we know from detection in individual cases, and these are based on, say, community studies of how many times - the estimate is based on how many times, you know, a year do you get sick and how many times a year is there a confirmed diagnosis.

And so, you end up with a huge number of cases only a small fraction of which have a confirmed diagnosis, looking at sporadic illness.

In terms of outbreaks, you're right. The 52% is probably an underestimate of the role of norovirus in outbreaks.

And in part in fairness to the folks on the bacterial side of things; part of that is due to improvements in, you know, sanitation and inspection and (things) - meat processing, things have actually reduced the number of foodborne (unintelligible).

(Wendy Zacowits): We can take one more question from the audience.

Coordinator: Okay. If anyone has one, please press star-1.

I'm showing no further questions.

(Wendy Zacowits): Right.

Well, if you have any questions you would like to ask Dr. Monroe off the phone, you may email your question to (neoffice@nltn.org). Dr. Monroe will answer your questions by email. Again, that email address is (neoffice@nltn.org).

I would like to remind all of the participants listening to register and complete an evaluation form by March 15, 2006. When you have completed the registration and evaluation form, we will be able to print your continuing education certificate.

The directions for this are on your confirmation letter and the general handouts.

Documenting your participation helps us continue to bring high-quality, cost-effective training programs in a variety of formats.

This concludes our program. Our next teleconference will be on March 15. The topic is avoiding diagnostic dilemmas in routine rabies testing.

The cosponsors of today's program would like to thank our speaker Dr. Steve Monroe. Thank you for joining us. I hope that all of you will consider joining us for our future programs and that you will make the National Laboratory Training Network your choice for laboratory training.

From the Arizona Department of Health Services in Phoenix, Arizona, this is (Wendy Zacowits). Have a great day.

END